REMARKS

I. Status of the Claims

Claims 8-13 are pending in the present application. Claims 1-7 and 14-20 have been withdrawn from consideration as a result of Applicants election, with traverse, of Group III claims in response to the restriction requirement dated February 4, 2003. Claims 9 and 10 have been amended to conform with Applicants election of the lysosomal storage disorder Galactosialidosis and protective protein/cathepsin A (PPCA), the corresponding enzyme which is deficient in this disorder, in response to this election of species requirement.

Applicants maintain their traversal of this restriction requirement. Reconsideration and withdrawal of this restriction requirement is respectfully requested for the reasons set forth in the response submitted February 13, 2003. Should this restriction requirement be maintained, Applicants reserve their right to file a petition to the Commissioner under 37 C.F.R. §§1.144 and 1.181 at any time until after final action or allowance of the elected claims to review the propriety of this requirement.

Claim 8 represents a genus claim linking species claims which must be examined with the elected invention in accordance with linking claim practice as set forth under MPEP §809. Further in accordance with linking claim practice, subject matter currently withdrawn from consideration as a result of the restriction requirement must be rejoined if this linking claim is found to be allowable.

II. Nonfinal Status of the Office Action

The summary page of the present Office Action indicates that it is final. However, as the first office action on the merits of an original application it would clearly be premature to make this a final Office Action (see 37 C.F.R. §1.113; MPEP §706.07). Further, no mention of finality

is mentioned in the Office Action itself. Therefore, the indication of finality on the summary page appears to represent an unintentional error. To the extent that the indication of finality was intentional, Applicants respectfully request withdrawal of finality as premature.

III. The Claim Objection Has Been Addressed

On page 2 of the Office Action claims 9 and 10 have been objected to because they include nonelected subject matter. Applicants have amended claims 9 and 10 herein to comply with the required election of species, thereby rendering this objection moot. Reconsideraton and withdrawal of this objection is therefore respectfully requested.

Should linking claim 8 be found allowable, Applicants reserve the right to amend claims 9 and 10 to recapture subject matter that is currently excluded by the election of species imposed by the Examiner in accordance with standard linking claim practice under MPEP §809.

IV. The Specification Provides a Sufficient Written Description of the Claimed Subject Matter

On page 3 of the Office Action the Examiner has rejected claims 8-13 under 35 U.S.C. §112, first paragraph, asserting that the claims contain subject matter which was not sufficiently described in the specification. Applicants respectfully traverse this rejection.

The basis for this rejection appears to be the absence of structural recitation of all of the proteins described in the specification which can be produced in accordance with the methods taught and combined with a pharmaceutically acceptable carrier to create the claimed pharmaceutical compositions. However, the structure of these proteins were well known in the art before the priority filing date of the present application and references to scientific literature and/or Genbank accession numbers disclosing such structures has been provided in the specification (*See*, in particular, Table 1). As such, these structures do not need to be included in the specification and in fact are preferably omitted according to *Hybridtech, Inc. v. Monoclonal*

Antibodies, Inc. 231 USPQ 81, 94 (Fed. Cir. 1986)("a patent need not teach, and preferably omits, what is well known in the art.").

The present invention is based upon the application of a particular method of protein production to a known group of proteins in order to produce novel and unobvious compositions that are uniquely suited to treat lysosomal storage disorders. The invention can be applied to any given protein useful for treating a lysosomal storage disorder, regardless of its structure. Applicants do not rely upon the primary structure; i.e. the amino acid sequence, of any of these proteins to impart patentability upon the claimed compositions. Instead Applicants properly rely on the knowledge of these structures to supplement the description of the novel and unobvious aspects of the invention in the specification. Recitation of the primary structure of each member of this group of proteins would be redundant to knowledge available in the prior art and is not necessary.

Reconsideration and withdrawal of this rejection is respectfully requested based on the reasons set forth above.

V. The Claimed Subject Matter is Enabled

On pages 3 and 4 of the Office Action the Examiner has rejected claims 8-13 under 35 U.S.C. §112, first paragraph, asserting that the claimed subject matter is not described in the specification is such a way as to enable one skilled in the art to make and/or use the invention. Applicants respectfully traverse this rejection.

Applicants initially note that the Examiner does not provide any basis for disputing the ability of the skilled artisan to produce the claimed pharmaceutical compositions. Nor does the Examiner provide any basis for disputing the ability of the skilled artisan to administer such compositions by conventional techniques. Rather, this rejection is based solely on skepticism regarding whether administration of the claimed compositions will work; i.e. whether the compositions will be safe and effective for treating the identified conditions. Thus this rejection

appears to be based more on concerns related to the utility of the claimed compositions when administered rather than concerns related to the ability to actually make and administer these compositions. As such the guidelines for examining applications for satisfaction of the utility requirement are applicable here and should be followed. Under these guidelines, Applicants respectfully submit that the claimed pharmaceutical compositions clearly have specific, substantial and credible utility.

A. Analysis of the *Wands* Factors

Turning to the specific reasons for this rejection, the Examiner appears to assert that undue experimentation would be required to practice the invention based on an analysis of factors enumerated in *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988). Applicants address each of these factors as applied to the claimed invention below to show that undue experimentation is not required.

(1) Quantity of experimentation necessary

The present invention utilizes a conventional protein production technique (baculovirus vector expression in insect cells) to produce proteins with a known association with lysosomal storage disorders. Many of these proteins have been produced in the prior art by other techniques and administered to humans to treat various lysosomal storage disorders. As a result, virtually no experimentation is needed to produce and administer a pharmaceutical composition according to the present invention. The only step where experimentation is involved at all is in the testing of such compositions for safety and efficacy to confirm their utility. This type of testing is routine in the art and can be accomplished using conventional techniques.

The Examiner asserts that the experimentation necessary to practice the invention "entails searching for any protein of any structure and function . . . ". Applicants disagree. Practice of the invention does not involve searching for any protein of any structure and function. The

proteins which can be produced as pharmaceutical compositions according to the invention are identified in the specification; no searching is involved. The only experimentation involved is routine safety and efficacy testing as noted above. While the Examiner asserts that experimentation which is not routine is involved, Applicants cannot determine what non-routine experimentation is considered by the Examiner to be necessary to practice this invention.

(2) The amount of direction or guidance presented

The specification describes the process of making and using the claimed pharmaceutical compositions in detail, with appropriate reliance on, and reference to, information already present in the prior art and available to the skilled artisan. The Examiner has not asserted any deficiencies with respect to this factor.

(3) The presence or absence of working examples

The specification provides specific support for the use of PPCA produced in insect cells to treat Galactosialidosis in an animal model of this protein deficiency. Applicants expect that additional support will be available in the near future in the form of published research from the inventors with respect to (a) neuraminidase and Sialidosis, and (b) beta-glucosidase and Gaucher Disease.

Applicants also note that exemplary support is unnecessary for the skilled artisan to understand and appreciate how to make and use the claimed compositions. These compositions comprise known proteins which are made using a conventional technique. These proteins were contemplated in the prior are to be useful for treating lysosomal storage disorders. In fact, many of the subject proteins have been made by different processes and used for this purpose.

(4) The nature of the invention

Applicants have taught how to make proteins already contemplated as therapeutic agents for treating lysosomal storage disorders in a subtly different form using a conventional method that had been used in the past for other purposes. As such this invention may be considered a simple improvement upon the state of the art. The simple nature of the invention and its use of information and conventional techniques available in the prior art weighs heavily in favor of enablement. This factor was not addressed in the Office Action.

(5) The state of the prior art

The state of the art relative to the claimed invention as of the priority filing date of September 28, 2001 was high. At that time, the specific protein deficiencies responsible for the various lysosomal storage disorders identified in the specification were well known. The approach of administering these proteins to individuals suffering from these disorders to overcome their particular deficiency was also well known, with a number of such proteins in human clinical trials (see specification at pages 6-8). In addition, while baculovirus expression in insect cells had not been appreciated as useful for producing proteins to treat humans with lysosomal storage disorders at the time of the invention, this expression method had been used to produce other proteins for administration to humans. For example, experimental vaccines produced via baculovirus expression in insect cells have been used in humans for HIV (Ratto-Kim, S. *et al., J. Infect. Dis. 179(2):* 337-344 (1999); copy attached as Exhibit A), influenza (Lakely, D.L. et al., J. Infect. Dis. 174(4): 838-841 (1996); copy of abstract attached as Exhibit B), and malaria (Herrington, D.A. et al., Vaccine 10(12): 841-846 (1992); copy of abstract attached as Exhibit C).

The particularly well advanced state of the prior art with respect to the invention is yet another factor that weighs in favor of enablement. Considering the state of the art at the time of this invention, the Examiner's skepticism regarding the utility of the claimed pharmaceutical

compositions is difficult to understand. Applicants are not claiming the use of completely new and unique therapeutic agents. Instead, Applicants have taught how to make proteins already contemplated as therapeutic agents for treating their associated lysosomal storage disorders in a subtly different and better form using an existing methodology.

(6) The relative skill of those in the art

The skill level in the field of producing, testing and administering proteins as pharmaceutical compositions is very high, typically involving Ph.D. and M.D. level personnel with several years of post-graduate training. This high relative skill level in the art further supports enablement of this invention.

(7) The predictability or unpredictability of the art

The present invention is based upon the production of known proteins using a conventional method that had been used in the prior art to produce other proteins for use in humans. The proteins produced are made into pharmaceutical compositions by conventional methodology to treat lysosomal storage disorders associated with a know deficiency in each of these proteins. This approach is highly predictable.

Concerns regarding the safety and/or efficacy of such polypeptides in humans are unsupported and improper. The Examiner does not provide any scientific rationale to support concerns that the claimed compositions are unsafe or ineffective. The only basis given for such concerns appears to be the absence of actual clinical data providing conclusive proof of the safety and efficacy of the claimed compositions. However, human testing is not required to satisfy utility and enablement requirements for human pharmaceuticals. *In re Brana,* 34 USPO 2d 1436 (Fed. Cir. 1995). Given the state of the art at the time of the invention as described above, there appears to be no basis for the skilled artisan to doubt the utility of the claimed pharmaceutical compositions.

(8) The breadth of the claims

The Examiner asserts that the claims encompass "any polypeptide of any structure and function to be used for treating any lysosomal storage disorder". Applicants would agree that the claims encompass any polypeptide that is contemplated to be useful for treating a lysosomal storage disorder. Such a claim scope is warranted given that the invention may be routinely applied to any polypeptide contemplated for such use.

B. The Rejection as Applied to the Elected Species

The nonenablement assertions are even weaker when applied to the specifically elected lysosomal storage disorder Galactosialidosis and corresponding enzyme which is deficient in this disorder, protective protein/cathepsin A (PPCA). Beside the extensive support from the state of the art in treating lysosomal disorders, the specification provides specific support for the use of PPCA produced in insect cells to treat Galactosialidosis in an animal model of this protein deficiency.

Applicants further note that the anticipation rejection made in the present Office Action contradicts the enablement rejection as applied to PPCA and Galactosialidosis. In that rejection, the Examiner asserts that the claims are anticipated with respect to Galactosialidosis and PPCA by WO 00/39150 (Sharp). Since a reference must be enabling in order to be anticipatory, the Examiner effectively asserts that this prior art reference is enabling for the claimed subject matter. However, this reference suffers from the same deficiencies the Examiner has alleged against the present specification to support his nonenablement assertion. Applicants submit that it is not logical to simultaneously assert that the specification is not enabling but that Sharp reference is enabling with respect to the presently claimed invention.

C. Summary

The claimed pharmaceutical compositions are made using conventional techniques as applied to known proteins and are fully enabled. These compositions are contemplated for the same therapeutic uses as contemplated in the prior art for these proteins produced by other methods. Reconsideration and withdrawal of this rejection for the reasons set forth above is therefore respectfully requested.

VI. Composition Limitations Inherent in the Process Used Cannot Be Ignored

On page 5 of the Office Action the Examiner has rejected claims 8-13 under 35 U.S.C. §102(a) asserting that the claimed subject matter is anticipated by Sharp, J.D., international application no. PCT/US99/31158, published as WO 00/31950. Applicants respectfully traverse this rejection.

In making this rejection, the Examiner admits to essentially ignoring the process used to make the protein component of the claimed pharmaceutical composition. The basis given for ignoring the process used is MPEP §2113, which describes examination of product-by-process claims.

MPEP §2113 does not support complete disregard for the process used to make a product in a product-by-process claim. Instead, it clarifies that patentability of such a claim depends on the character of the product produced, a proposition with which Applicants can readily agree. In this case Applicants have described, to the extent possible, the unique character of proteins produced in insect cells regarding post translational modifications, particularly with respect to their glycosylation pattern and more particularly with respect to exposed mannose residues (see, e.g., specification at page 2, lines 10-21 and page 14, lines 7-10) . Since these unique characteristics do not easily fit into the form of a claim limitation, Applicants have chosen to use the product by process claim format that is designed for this sort of situation. When this format

is used, unique characteristics of the product resulting from the process used cannot simply be ignored and afforded no patentable weight.

Sharp fails to teach a pharmaceutical composition comprising a protein that has the unique post-translational modifications associated with insect cell production and thus fails to anticipate the present product claims as properly construed to include limitations inherent in the insect cell production process used to make them. Reconsideration and withdrawal of this rejection on this basis is therefore respectfully requested.

VI. Conclusion

In view of the amendment to the claims and the remarks above, it is believed that the Examiner may properly withdraw the objection to claims 9-10 and rejection of the claims under 35 U.S.C. §102(a), and §112, first paragraph.

Having now fully responded to the Examiner's rejection of the claims, Applicants respectfully submit that the present application is in condition for allowance and earnestly solicit early notice of such favorable action. No fee is believed to be required for consideration of this submission. If applicants are incorrect and a fee is required the Commissioner is hereby authorized to charge such fee to Deposit Account No. 501968.

Respectfully submitted,

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Repeated Immunization with Recombinant gp160 Human Immunodeficiency Virus (HIV) Envelope Protein in Early HIV-1 Infection: Evaluation of the T Cell Proliferative Response

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This longitudinal study was designed to evaluate cellular immunity in early-stage, asymptomatic human immunodeficiency virus (HIV)-1–infected persons (CD4 cell count, >400/mm^3; median, 625/mm^3) who were immunized with either recombinant (r) gp160 or placebo every 2 months for 5 years. Proliferative responses were assessed against rgp160, rp24, and a panel of recall antigens and mitogens. Despite good reactivity to recall antigens, at baseline ~33% had proliferative responses to gp160, and ~42% showed p24 gag responses. There was no statistical difference between vaccine and placebo groups for antigens or mitogens. After 1 year, ~73% of the subjects in the vaccine arm had new or boosted responses to gp160, versus ~18% in the placebo arm. Statistical significance was maintained throughout the study. Recurrent vaccination with recombinant gp160 was proven to be persistently immunogenic, increasing significantly the ability of HIV-1–infected persons to mount new proliferative responses to the vaccine.

Cellular immune function is likely to play an important role in control of human immunodeficiency virus type 1 (HIV-1) infection. However, T cell proliferative responses to HIV antigens in vitro in seropositive patients are typically absent or small in magnitude [1–5]. In particular, in the early stages of disease, there is little proliferation to HIV-1 envelope proteins, despite a vigorous antibody response to most HIV-1 proteins [5]. It has been reported that in vitro proliferative responses to recall antigens and delayed-type hypersensitivity reactions to routine microbial recall antigens are also weaker in early-stage HIV-1–seropositive patients compared with normal controls [3, 4, 6, 7]. Presumably related to numerical or functional CD4 lymphocyte decline, infected patients gradually, but progressively, lose their ability to respond to recall antigens and mitogens [5, 8, 9].

Previous studies demonstrated that it is possible to modify the immunologic response to HIV-1 proteins in infected persons [10–13]. After a series of vaccinations with a recombinant HIV-1 envelope protein (rgp160) or a whole inactivated HIV-1 preparation, HIV-1–infected persons were able to mount new proliferative responses to envelope proteins and/or new delayed-type hypersensitivity responses, as well as novel epitope-specific antibody responses, compared with natural history cohorts [10]. The same recombinant protein was also tested in HIV-1–seronegative persons in phase I trials and was shown to be safe and immunogenic [14–17].

These early encouraging studies led the way to a double-blind placebo-controlled phase II trial using the rgp160 in early-stage HIV-1–infected patients. The aim of the study was to evaluate the safety and potential efficacy of therapeutic vaccination and possibly to correlate any new or boosted immune responses with a better clinical outcome; however, there were no clinically beneficial effects associated with the vaccination compared with placebo (Birx DL, unpublished data).

In this study, we compared the proliferative response to HIV-1 antigens, recall antigens, and mitogens between the rgp160 vaccine and placebo groups during the 5-year trial.

Materials and Methods

Patient population. A multicenter double-blind placebo-controlled safety and efficacy trial was conducted in two phases. One

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The research was approved by the Tri-service Walter Reed Army Medical Center, National Navy Medical Center, and Wilford Hall Medical Center Human Use Committee/Institutional Review Board. All subjects enrolled in the study voluntarily agreed to participate and gave written informed consent.

The views and opinions expressed herein are those of the authors and are not construed as official or as reflecting the views of the US Army, US Navy, or the Department of Defense.

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The Journal of Infectious Diseases 1999;179:337–44

0022-1899

hundred forty-one HIV-1–infected persons 18–60 years of age with CD4 cell counts >400/mm^3, who were eligible for health care within the US Department of Defense (DOD) health care system, were enrolled and followed for 14–18 months to evaluate immunogenicity (phase IIA). Patients were evaluated every 2 months and received injections of vaccine or placebo at three DOD clinical sites: Walter Reed Army Medical Center, Washington, DC (WRAMC), National Navy Medical Center, Bethesda, Maryland (NNMC), and Wilford Hall Medical Center, San Antonio (WHMC). Subsequently, 467 HIV-1–infected persons were enrolled in the trial from both the DOD health care system and civilian sites (phase IIB/IIC). A total of 608 subjects were followed for 3–5 years (phase IIA mean days on trial, 1563 [range, 58–1792]; phase IIB/IIC mean days on trial, 1114 [range, 170–1354]). Patients were randomly assigned in groups of 4 to the vaccine or placebo arm and received VaxSyn (160 μg) or placebo (aluminum phosphate) as intramuscular injections on days 0, 7, and 30 and every 2 months thereafter for up to 62 months. In this study, only the original phase IIA volunteers (36 subjects from WRAMC, 40 from NNMC, and 65 from WHMC) were evaluated because of their longer enrollment. Sample size decreased over time because of discontinued patients and a reduction in the number of analyses done in the standard proliferative assay because of insufficient numbers of cells (table 1).

Sample collection and preparation. Whole blood was collected and heparinized before immunization, and after ficoll separation, peripheral blood mononuclear cells (PBMC) were used in lymphoproliferative assays. Proliferation assays were done with fresh PBMC for patients whose clinical visits were at local sites (WRAMC and NNMC). PBMC collected at WHMC, after ficoll separation, were cryopreserved and shipped to the Rockville laboratory, where they were assayed at a later date.

Medium, antigens, and mitogens. Lymphocytes were cultured in complete media, composed of RPMI 1640 (Gibco, Grand Island, NY) supplemented with L-glutamine, 4 m*M*, penicillin, 100 U/mL, streptomycin, 100 μg/mL, HEPES, 25 m*M*, and 10% heat-inactivated normal human AB serum (ABI, Columbia, MD).

The baculovirus-expressed recombinant proteins gp160 (NL4-3 clone, identical to the vaccine used in the trial) and p24, as well as baculovirus control protein (bcp), were obtained from MicroGeneSys (Meriden, CT). Tetanus toxoid, diphtheria toxoid (Connaught, Willowdale, Canada), and *Candida albicans* dialyzed antigen (Hollister Stier, Spokane, WA) were used as recall antigens. Pokeweed mitogen (PWM), phytohemagglutinin (PHA), and concanavalin A (ConA) were obtained from Sigma (St. Louis).

Lymphoproliferative assays. PBMC proliferative responses to antigens and mitogens were measured by incubating 10^5 cells/well in 96-well U-bottom plates (Costar, Cambridge, MA) with serial antigen concentrations of gp160 (6.2, 3.1, 1.5, 0.8, and 0.4 μg/mL), p24 (25 and 12.5 μg/mL), bcp (1 and 0.1 μg/mL), tetanus toxoid (6.5, 3.2, and 1.6 Lf units), diphtheria toxoid (23, 11.5, and 5.8 Lf units), and *C. albicans* antigen (1:200, 1:400, and 1:800) and in separate plates with serial dilutions of PHA (2, 1, and 0.5 μg/mL), PWM (2.5, 1.2, and 0.6 μg/mL), and ConA (20, 10, and 5 μg/mL). After 3 days of incubation with the mitogens and 7 days with the antigens, cells were pulsed with 1.56 μCi/well [^{3}H]thymidine for 18 h, harvested by use of the Skatron harvesting system (Skatron Instruments, Sterling, VA), and counted in a beta counter (BetaPlate, model 1205; Wallac, Uppsala, Sweden).

Antigens were prioritized for plating in the event that specimens did not yield sufficient numbers of cells. The data are expressed as a lymphocyte stimulation index (LSI) (mean counts per minute of stimulated cells/mean counts of unstimulated cells) to define antigen

Table 1. Nos. of subjects assayed for proliferative response.

	Baseline		Year 1		Year 2		Year 3		Year 4		Year 5	
Antigen or mitogen, arm	A	B	A	B	A	B	A	B	A	B	A	B
gp160												
Vaccine	37	31	37	31	37	30	34	24	26	20	20	23
Placebo	36	35	36	35	36	34	35	29	28	26	15	25
p24												
Vaccine	37	23	37	31	37	29	34	23	26	19	20	22
Placebo	35	29	36	35	36	33	35	28	28	26	15	24
Phytohemagglutinin												
Vaccine	37	30	37	31	37	27	34	—	25	—	19	—
Placebo	36	35	36	35	36	26	35	—	27	—	15	—
Pokeweed mitogen												
Vaccine	37	30	37	31	37	30	34	22	26	17	19	16
Placebo	36	34	36	35	36	32	35	27	27	20	15	12
Concanavalin A												
Vaccine	37	30	36	31	36	10	34	—	25	—	19	—
Placebo	36	34	36	35	36	12	35	—	27	—	15	—
Diphtheria toxoid												
Vaccine	37	30	36	31	37	29	34	24	26	20	20	22
Placebo	36	35	36	35	36	33	35	29	28	26	15	24
Tetanus toxoid												
Vaccine	37	30	37	31	37	30	34	24	26	21	20	22
Placebo	36	35	36	35	36	34	35	29	28	26	15	25
Candida albicans												
Vaccine	37	—	36	—	36	—	34	—	25	—	19	—
Placebo	36	—	36	—	36	—	35	—	27	—	15	—

NOTE. Groups A and B, fresh or cryopreserved peripheral blood mononuclear cells, respectively. —, not done.

specificity. Samples were arbitrarily designated as positive if the LSI was ≥5.

Data analysis. The sample set was divided into 2 groups: group A, representing samples assayed fresh, collected at WRAMC and NNMC, and group B, representing samples assayed from cryopreserved PBMC collected at WHMC. These groups were then divided into vaccine and placebo recipients.

For each subject, the mean LSI value of the maximal proliferative response assessed for each antigen and mitogen was calculated within each time period (baseline and year 1, 2, 3, 4, and 5). This mean value for each subject, time period, and antigen or mitogen was the observation used for comparisons. For each variable, gp160, p24, PHA, PWM, ConA, diphtheria toxoid, tetanus toxoid, and *C. albicans* antigen, a Kruskal-Wallis nonparametric test was done for each of the time periods, to evaluate differences between mean proliferative responses in the vaccine and placebo groups. A χ^2 test was used to evaluate the difference in the percentage of responders (LSI ≥5) between groups.

Results

Comparison of the proliferative response of fresh versus cryopreserved PBMC. Because of logistical issues involving distance from the laboratory, cryopreserved PBMC were used in the proliferation assay for about half of the cohort. Data were analyzed and compared to assess any differences between the two techniques. Although there were occasional statistically significant differences in the comparative analyses (data not shown), fresh PBMC (group A) results were substantially similar to those with cryopreserved PBMC (group B). In general, values obtained from frozen samples were lower than those from fresh, but the percentage of responders was consistent between sites, as well as the relationship between vaccine and placebo. No trend was observed that might have resulted in unintentional bias during the comparison of proliferative responses between the vaccine and placebo groups.

Proliferative responses at baseline: effects of randomization. HIV-1–infected subjects were block-randomized in groups of 4 to receive vaccine or placebo after prescreening for study entry criteria. Blood was drawn three or four times before the patient was enrolled and immediately before the first injection (day 0) to establish a baseline for comparison. Antigen and mitogen proliferation were tested in all of the preenrollment and day 0 samples. Results (expressed in LSI) were averaged to constitute the baseline values. All of the samples were sent to the proliferation laboratory with sequential identification numbers; therefore, no connection between identification numbers and individual patients was possible.

Baseline proliferation was similar between the vaccine and placebo recipients, and no statistical differences in responsiveness were found for any of the antigens or mitogens tested (table 2). Median baseline T cell proliferative responses to HIV-1 antigens were poor (figures 1, 2); only about one-third of the subjects were able to recognize gp160, and ~30%–50% were able to recognize p24 (table 2).

The proliferative response was 97%–100% positive for all three mitogens used in the study. Mean baseline positive responses to recall antigens were ~74%, 62%, and 45%, respectively, for tetanus toxoid, diphtheria toxoid, and *C. albicans* antigen. Because the study population was largely composed of active-duty military personnel who received routine booster vaccinations more frequently compared with civilians, the tet-

Table 2. Percentage of responders in each group by study year.

	Baseline		Year 1		Year 2		Year 3		Year 4		Year 5	
Group, antigen or mitogen	Vaccine	Placebo	Vaccine	Placebo	Vaccine	Placebo	Vaccine	Placebo	Vaccine	Placebo	Vaccine	Placebo
Group A												
gp160	30[a]	39	89	33[a]	89	14[a]	79	23[a]	50	46	35	0[a]
p24	51	54	57	64	70	56	35	34	35	50	25	0
bcp	0	0	0	0	0	0	12	6	0	0	0	0
PHA	100	100	100	100	100	100	100	100	92	93	89	93
PWM	97	97	100	97	100	100	100	100	100	100	89	100
ConA	100	100	100	100	100	100	100	100	100	100	95	100
Tetanus toxoid	70	81	70	92[a]	78	92	82	89	77	75	20	47
Diphtheria toxoid	70	69	67	78	49	67	59	77	38	43	10	13
Candida albicans	43	47	39	61	44	53	50	60	48	48	11	7
Group B												
gp160	32	31	87	34[a]	67	32[a]	63	21[a]	45	4[a]	48	0[a]
p24	35	28	45	31	38	42	30	25	11	8	9	8
bcp	6	3	3	0	10	3	4	4	0	0	9	4
PHA	100	100	100	100	96	100	—	—	—	—	—	—
PWM	100	100	100	100	90	100	100	96	94	100	100	92
ConA	100	100	100	100	100	100	—	—	—	—	—	—
Tetanus toxoid	83	63	71	71	67	79	63	69	29	62	27	32
Diphtheria toxoid	57	54	58	54	38	39	33	62	30	27	23	29
C. albicans	—	—	—	—	—	—	—	—	—	—	—	—

NOTE. Groups A and B, fresh or cryopreserved peripheral blood mononuclear cells, respectively. Responses were considered positive when lymphocyte stimulation index was ≥5. PHA, phytohemagglutinin; PWM, pokeweed mitogen; ConA, concanavalin A; bcp, baculovirus control protein; —, not done.

[a] χ^2, $P \leq .01$.

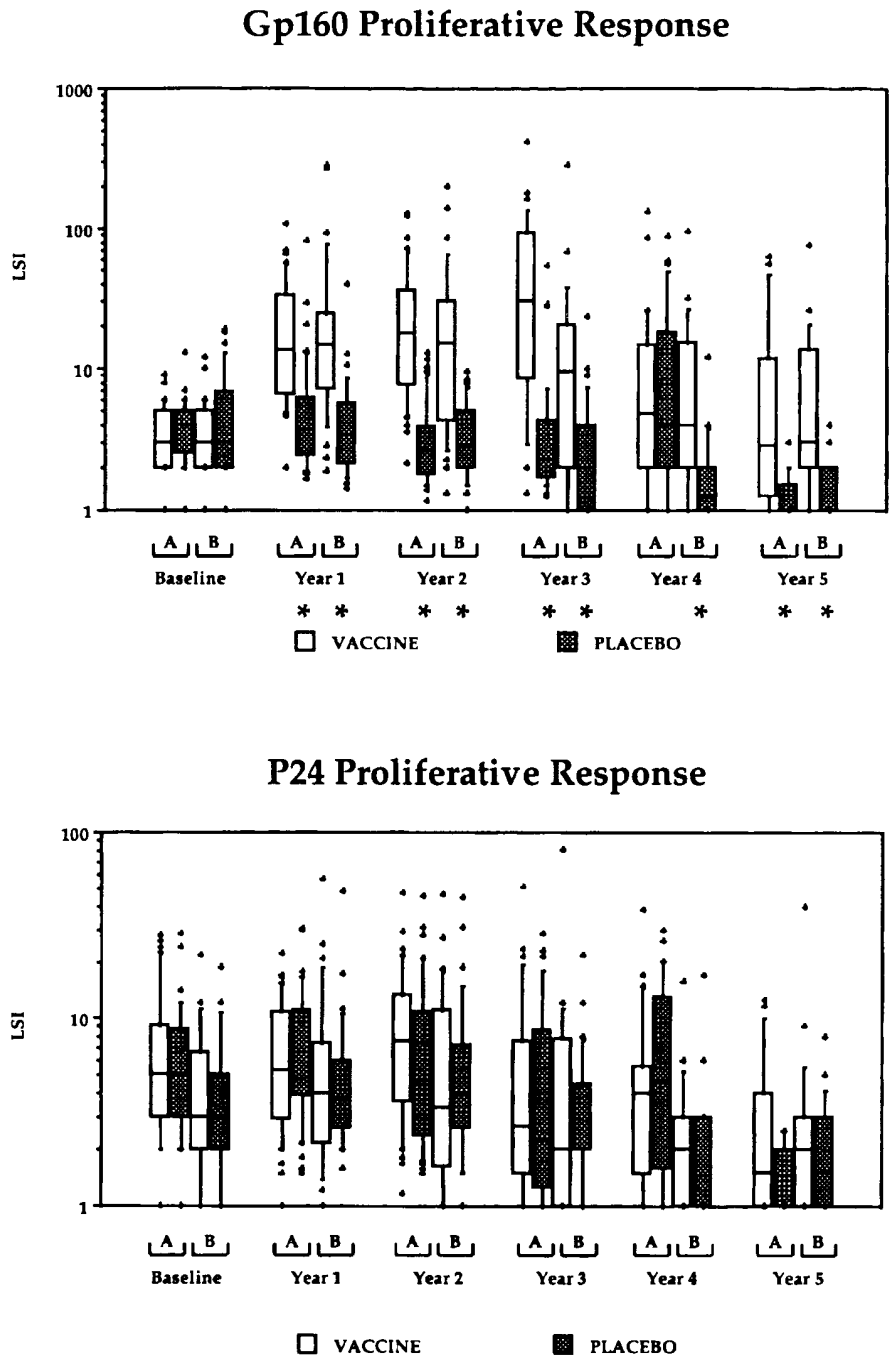

Figure 1. Box plot graph representing mean lymphocyte stimulation index (LSI) over time for responses to HIV-1 proteins. * Statistical difference between vaccine and placebo group ($P < .01$). Groups A, B = fresh and cryopreserved peripheral blood mononuclear cells, respectively.

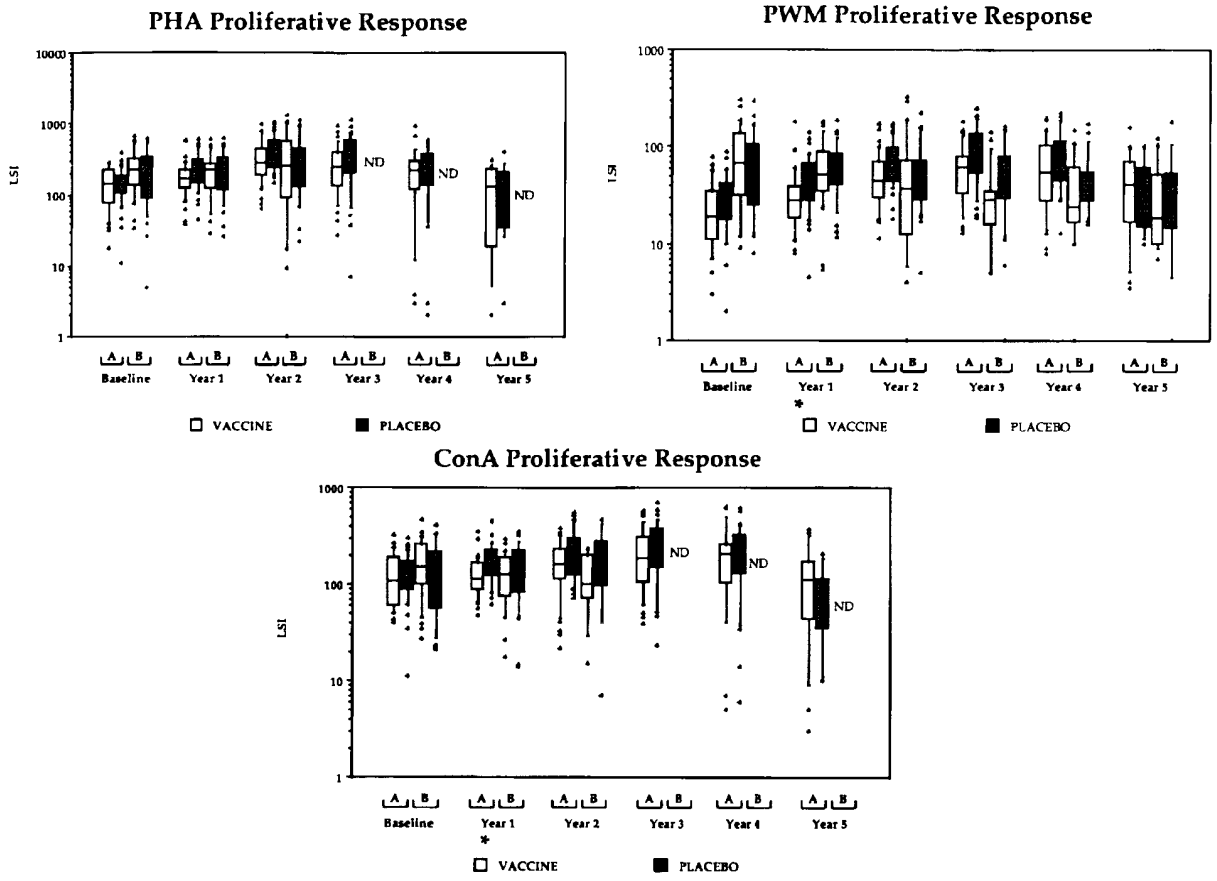

Figure 2. Box plot graph representing mean lymphocyte stimulation index (LSI) over time for responses to mitogens (PHA, phytohemagglutinin; PWM, pokeweed mitogen; ConA, concanavalin A). * Statistical difference between vaccine and placebo group ($P < .01$). Groups A, B = fresh and cryopreserved peripheral blood mononuclear cells, respectively.

anus toxoid responses were higher at baseline than previously reported [11].

Comparison of proliferative responses between vaccine and placebo arms during the 5-year follow-up. The proliferative response to the rgp160 developed rapidly in the vaccine arm, reaching statistical significance compared with the placebo arm at day 30, after the first two injections (data not shown). This statistical significance was maintained throughout the study for both mean LSI and percentage positive in group B (figure 1; table 2), and for all time points but year 4 for group A (figure 1; table 2). To better characterize the response to rgp160, yearly average LSI for each subject was defined as new/boost if the subject had a >2-fold increase from his or her baseline value, as well as LSI >5. After 1 year, 78% and 68% (group A and B, respectively) of the subjects in the vaccine arm developed a new/boost response, while in the placebo group, only 22% and 14%, respectively, had a new/boost response (table 3). These data confirm previous observations on the immunogenicity of this recombinant vaccine.

Table 3. Comparison of new/boost responses between vaccine and placebo arms.

	Vaccine		Placebo	
	Group A	Group B	Group A	Group B
Year 1	78	68	22	14
Year 2	84	63	8	21
Year 3	79	54	9	14
Year 4	42	38	43	0
Year 5	30	48	0	0

NOTE. Groups A and B, fresh or cryopreserved peripheral blood mononuclear cells, respectively. Data are expressed as % who had >2-fold increase from their baseline lymphocyte stimulation index (LSI) to at least LSI >5. All comparisons are yearly average vs. baseline LSI.

Because the recombinant gp160 used as vaccine in this trial was a baculovirus-expressed recombinant protein produced in the cells of lepidopteran insects, bcp was tested in the proliferation assay to assess any sensitization to this contaminant. Baseline response to bcp was surprising, 3% for placebo and

6% for vaccine recipients in group B and 0 for group A (table 2). After 1 year, the percentage of responders to bcp did not substantially change, although by year 3 the proliferative response to bcp in group A was assessed at 12% of subjects in the vaccine and 6% of subjects in the placebo arm (table 2). These levels of response are concordant with levels observed in previous studies [10, 11, 14, 15].

The proliferative response to mitogens was optimal throughout the study (table 2); a slight decrease was observed in the response to PHA in group A, but no statistical difference was observed between the 2 groups. Responses to recall antigens demonstrated, in general, a trend toward greater LSI in both vaccine and placebo arms in the early years, followed by a decline below the baseline value by year 5 (table 2; figures 1–3). Proliferative response against p24 and PHA did not differ between the two arms for the duration of the study (figures 1, 2). Interestingly, there was a statistically higher LSI for the placebo recipients to PWM, ConA, diphtheria toxoid, and *C. albicans* antigen at year 1 and tetanus toxoid at year 1, 2, and 3 for group A (figures 2, 3). This effect resulted largely from an increase in LSI of these antigens and mitogens compared with baseline values. There was no difference in the mean background counts between the vaccine and placebo group over the 5 years of study (data not shown).

Discussion

It has been shown that early-stage HIV-1–infected persons sequentially immunized with a recombinant gp160 protein retain the functional ability to mount new cellular and humoral-mediated responses to that immunogen despite viral infection [10–12]. On the basis of these findings, a double-blind placebo-controlled phase II trial was conducted to determine the safety, immunogenicity, and efficacy of this vaccine. The present study

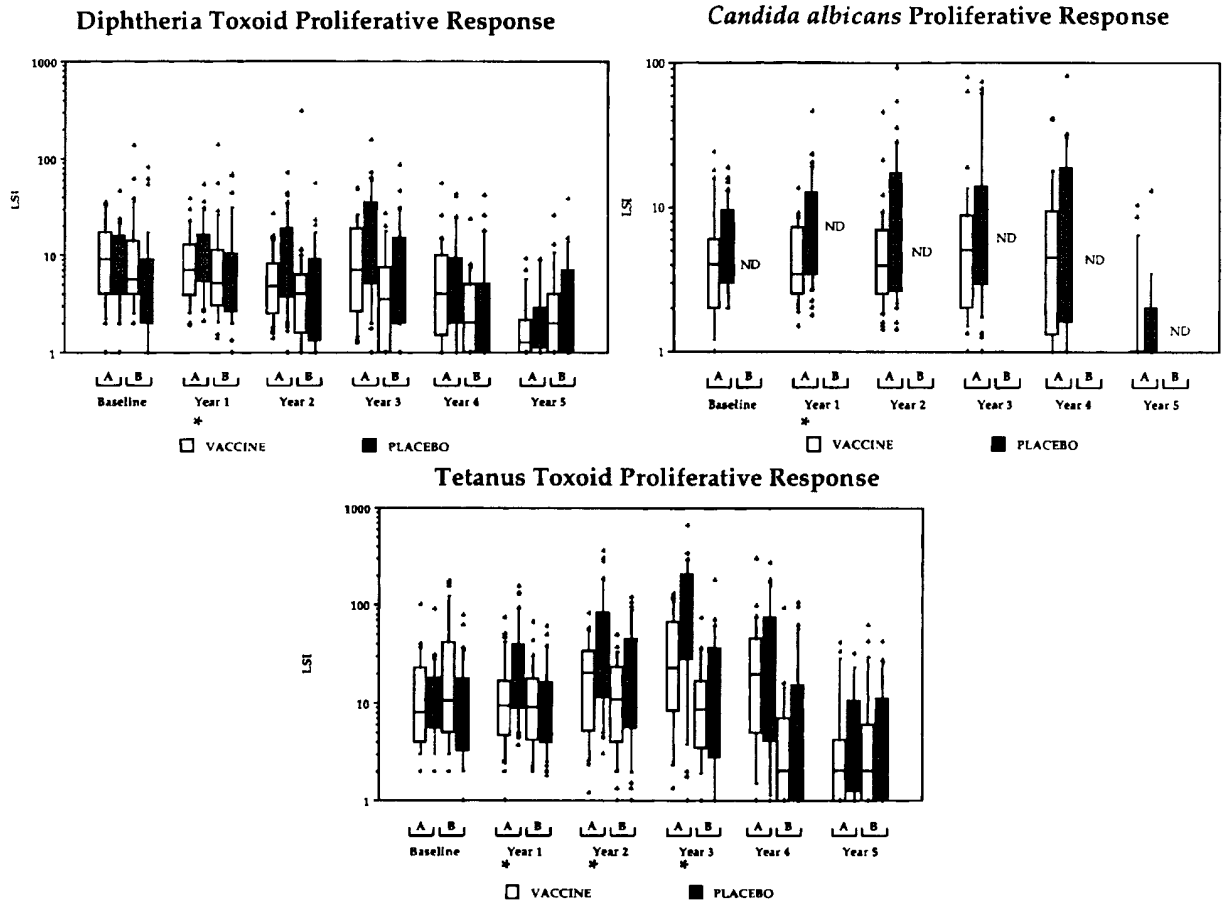

Figure 3. Box plot graph representing mean lymphocyte stimulation index (LSI) over time for responses to recall antigens. * Statistical difference between vaccine and placebo group ($P < .01$). Groups A, B = fresh and cryopreserved peripheral blood mononuclear cells, respectively.

evaluates the cellular immune response to recombinant gp160 during the 5 years of the phase II trial. The aims of this study were to monitor the evolution of the proliferative response to the vaccine, different mitogens, and recall antigens and to assess the effect of extended chronic antigenic stimulation of the immune system on the proliferative response of HIV-1–infected persons. Because of logistical problems, half of the proliferative responses to antigens and mitogens in this cohort were measured using cryopreserved PBMC.

The baseline responses to HIV-1 and recall antigens were similar, and no statistical difference was observed between the vaccine and placebo groups. These data are in agreement with other studies in which similar cohorts of patients were evaluated for their ability to proliferate to HIV-1 antigens [1, 11, 18, 19].

The response to the rgp160 vaccine was rapid and strong, with the vaccine group reaching statistically higher proliferation by day 30. Other studies of rgp160 have collected similar data in both HIV-1–infected and uninfected persons [11, 14–17], confirming the immunogenicity of this recombinant gp160 vaccine. The responses to rgp160 were maintained throughout the study, with some diminution in year 4 and 5, showing the ability of the immune system in HIV-1–infected persons to sustain a proliferative response. Although a strong cellular immune response to the HIV-1 envelope was maintained throughout the study compared with that in the placebo group, the repeated vaccinations were not associated with any clinical benefit. Vaccine and placebo arms showed no difference in CD4 cell decline, virus burden, study events related to development of symptoms, AIDS, or death (Birx DL, Sitz KV, unpublished data).

Because of the potential deleterious effects of repeated immunizations, it was important to monitor other cellular responses concomitantly [20, 21]. Proliferation to recall antigens and mitogens was routinely tested throughout the trial. Wahren et al. [11] reported that vaccination with gp160 improved the responses to recall antigens, such as tetanus toxoid, measles, and herpes simplex virus, and to PWM [11]. The data on T cell proliferative responses compiled in the 5 years of this phase II trial differ from those previously reported. In both vaccine and placebo groups, there were slight increases in the mean LSI during the first 2 years for most mitogens and antigens, followed by a decrease below baseline by year 5. Interestingly, compared with baseline, there was a trend toward enhanced proliferation within the placebo group. This trend resulted in statistically significant, albeit sporadic and transient, differences between the proliferative response of the vaccine and placebo groups to *C. albicans* antigen, ConA, PWM, diphtheria toxoid, and tetanus toxoid at various intervals during the trial. The clinical outcome between the 2 groups, however, was identical, so these small variations in the ability to proliferate to antigens or mitogens did not correlate with differences in disease progression.

Because of the number of tests done, the statistically significant differences between the 2 groups could be due to chance alone. Alternatively, there might be a normal variability in the ability to proliferate to the same antigens over time in populations, although if this were true, we should have observed this phenomenon occurring as a random event in both groups. It could also be possible that aluminum phosphate, as was received by the placebo group, accounts for the transient differences in response to recall antigens and mitogens. Since no data are available on the effect of chronic stimulation with adjuvants, this hypothesis is difficult to prove but, nonetheless, needs to be taken into consideration.

In conclusion, recurrent vaccination with recombinant gp160 was proven to be persistently immunogenic, increasing significantly the ability of HIV-1–infected persons to mount new proliferative responses to the vaccine. Concurrently, the proliferative responses to mitogens and recall antigens yielded sporadic and unsustained differences between the vaccine and placebo groups. There was little evidence that chronic immunization of this HIV-infected population caused degradation of proliferative responses to unrelated recall antigens or more rapid clinical deterioration.

Acknowledgments

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EXHIBIT B

1: J Infect Dis 1996 Oct;174(4):838-41 Related Articles, Books, LinkOut

Recombinant baculovirus influenza A hemagglutinin vaccines are well tolerated and immunogenic in healthy adults.

Lakey DL, Treanor JJ, Betts RF, Smith GE, Thompson J, Sannella E, Reed G, Wilkinson BE, Wright PF.

Department of Pediatrics, Vanderbilt University, Nashville, Tennessee 37232-2581, USA.

In a prospective double-blind trial, the reactogenicity and immunogenicity of recombinant baculovirus influenza A vaccines containing purified full-length hemagglutinin (HA) were compared with standard trivalent inactivated vaccine (TIV). The recombinant baculovirus influenza A vaccines (rHA0) were monovalent (containing 45 micrograms of A/Beijing/92[H3] and 15, 45, and 135 micrograms of A/Texas/91[H1]) and bivalent (containing 45 micrograms of both A/Beijing/92 and A/Texas/91). The bivalent rHA0 vaccine produced fewer local side effects than the TIV (50% vs. 88%, P = .003). The hemagglutinin inhibition (HAI) responses (defined as a > or = 4 increase in HAI) to A/Beijing rHA0 in the monovalent A/Beijing/92, the bivalent vaccine, and the TIV were 68%, 76%, and 46%, respectively (P = .086). Increasing doses of A/Texas rHA0 (15 micrograms [60%], 45 micrograms [69%], and 135 micrograms [76%]) and bivalent HA (76%) gave better immunologic responses to H1 than did TIV (31%; P = .003).

Publication Types:
- Clinical Trial
- Randomized Controlled Trial

PMID: 8843225 [PubMed - indexed for MEDLINE]

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EXHIBIT C

1: Vaccine 1992;10(12):841-6 Related Articles, Books, LinkOut

Safety and immunogenicity in volunteers of a recombinant Plasmodium falciparum circumsporozoite protein malaria vaccine produced in Lepidopteran cells.

Herrington DA, Losonsky GA, Smith G, Volvovitz F, Cochran M, Jackson K, Hoffman SL, Gordon DM, Levine MM, Edelman R.

Department of Medicine, University of Maryland, Baltimore 21201.

A recombinant Plasmodium falciparum circumsporozoite (CS) antigen (rPfCSA) was produced in insect cells using a baculovirus expression vector containing the entire CS gene. This near full-length CS antigen was adsorbed onto aluminium phosphate for use as a malaria vaccine. In a study of safety and immunogenicity, 20 volunteers were divided into four groups of five each and inoculated intramuscularly with 10, 100, 500 or 1000 micrograms of vaccine. Primary vaccinations were followed by two booster immunizations at 2 and 6 months. Three volunteers developed prominent local reactions manifested as tenderness, redness and swelling at the injection site following the second or third vaccination. All symptoms resolved spontaneously within 72 h. Postimmunization sera from six of 20 volunteers showed seroconversions as measured by Western blot, using rPfCSA as antigen. However, specific anti-CS protein antibody could not be detected by indirect immunoflourescence against intact sporozoites or by ELISA using rPfCSA or peptide to the repeat region. In addition, 18 of 20 volunteers developed antibody to baculovirus proteins as determined by ELISA and/or Western blot. Antigen-driven replication studies using peripheral blood mononuclear cells from vaccinees failed to detect proliferative responses specific to CS protein. This recombinant CS protein vaccine, as formulated, was minimally immunogenic in humans.

Publication Types:
- Clinical Trial
- Controlled Clinical Trial

PMID: 1455910 [PubMed - indexed for MEDLINE]